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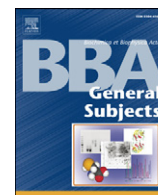

Review

Glutathione and infection ☆

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ABSTRACT

Background: The tripeptide γ-glutamylcysteinylglycine or glutathione (GSH) has demonstrated protective abilities against the detrimental effects of oxidative stress within the human body, as well as protection against infection by exogenous microbial organisms.
Scope of review: In this review we describe how GSH works to modulate the behavior of many cells including the cells of the immune system, augmenting the innate and the adaptive immunity as well as conferring protection against microbial, viral and parasitic infections. This article unveils the direct antimicrobial effects of GSH in controlling *Mycobacterium tuberculosis* (*M. tb*) infection within macrophages. In addition, we summarize the effects of GSH in enhancing the functional activity of various immune cells such as natural killer (NK) cells and T cells resulting in inhibition in the growth of *M. tb* inside monocytes and macrophages. Most importantly we correlate the decreased GSH levels previously observed in individuals with pulmonary tuberculosis (TB) with an increase in the levels of pro-inflammatory cytokines which aid in the growth of *M. tb*.
Major conclusions: In conclusion, this review provides detailed information on the protective integral effects of GSH along with its therapeutic effects as they relate to the human immune system and health.
General significance: It is important to note that the increases in the levels of pro-inflammatory cytokines are not only detrimental to the host due to the sequel that follow such as fever and cachexia, but also due to the alteration in the functions of immune cells. The additional protective effects of GSH are evident after sequel that follows the depletion of this antioxidant. This is evident in a condition such as Cystic Fibrosis (CF) where an increased oxidant burden inhibits the clearance of the affecting organism and results in oxidant-induced anti-protease inhibition. GSH has a similar protective effect in protozoans as it does in human cells. Thus GSH is integral to the survival of some of the protozoans because some protozoans utilize the compound trypanothione [$T(SH)_2$] as their main antioxidant. $T(SH)_2$ in turn requires GSH for its production. Hence a decrease in the levels of GSH (by a known inhibitor such as buthionine sulfoximine [BSO] can have adverse effects of the protozoan parasites. This article is part of a Special Issue entitled Cellular functions of glutathione.

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1. Introduction

Glutathione (γ-glutamylcysteinylglycine, GSH), an ubiquitous sulfhydryl-containing tripeptide produced by most mammalian cells, is the cells principle mechanism of eliminating reactive oxygen species (ROS) [1–3]. GSH is synthesized *de novo* in a two-step enzymatic process in which glutamine and cysteine are covalently linked by the heterodimeric enzyme γ-glutamylcysteine synthetase or glutamate-cysteine ligase (GCL) to form the product γ-glutamylcysteine (Fig. 1A). This is the rate limiting step in the synthesis of GSH, and cysteine is both the rate limiting reactant and the component that provides GSH with antioxidant activity, as cysteine's sulfhydryl bond is oxidized during the reduction of ROS [1–3]. In the second step of the reaction, γ-glutamylcysteine is bonded to glycine to form a complete GSH molecule (Fig. 1B). Using GSH as a substrate, glutathione peroxidase (GPx) detoxifies hydrogen peroxide, a potent source of ROS within the cell [1–3]. GPx performs the reduction of hydrogen peroxide to water, while linking two GSH molecules together via a disulfide bridge to form oxidized glutathione (GSSG) (Fig. 2A). GSSG is unable to perform antioxidant functions; however, GSH can be reclaimed from GSSG through the use of glutathione reductase (GSR) [1–3]. Utilizing NADPH as a co-factor, GSR performs the reduction of GSSG to GSH, and oxidation of NADPH to NAD^+ (Fig. 2B). Unfortunately, this GSH system can be overwhelmed if ROS are produced in excess. If this occurs the remaining excess free radicals begin to do damage to molecules essential to cellular homeostasis and metabolism, including proteins,

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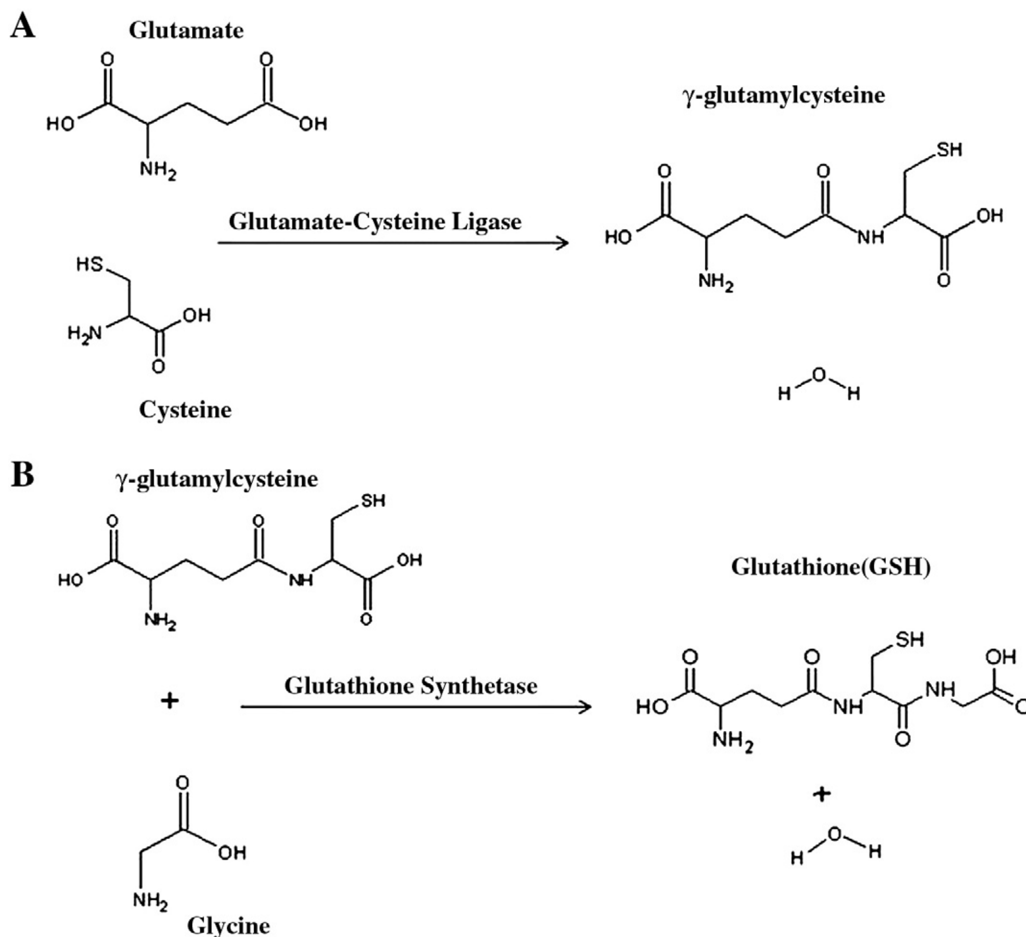

Fig. 1. A. Demonstrates *de novo* synthesis of GSH in twostep enzymatic process in which glutamine and cysteine are covalently linked by the heterodimeric enzyme γ-glutamylcysteine synthetase or glutamate-cysteine ligase (GCL) to form the product γ-glutamylcysteine. B: Demonstrates the second step in the reaction, γ-glutamylcysteine is bonded to glycine to form a complete GSH molecule.

nucleic acids, and lipids. This oxidative damage has real consequences for cellular functions.

2. GSH in prokaryotes

GSH is produced by most eukaryotes and is produced by some but not all prokaryotes. This important antioxidant is synthesized under the control of diverse systems depending on which prokaryote is being considered. In *Escherichia coli* (*E. coli*) oxidative stress induces the synthesis of GSH, through upregulation of two enzymes γ-glutamylcysteine synthetase and GSH synthetase. OxyR a transcription factor can sense and respond to oxidative stress. In its oxidized form it can activate transcription of promoters upstream of genes involved in resistance to oxidative damage and these include genes involved in GSH synthesis. Interestingly GSH is not required for growth of *E. coli* during logarithmic phase but is required for growth during stationary phase [4]. In another species of bacterium, *Salmonella* spp., it is the stringent response, or response to nutrient deprivation that upregulates GSH production [5]. ppGpp is produced in response to conditions of nutrient starvation, which in turn activates a response regulator that upregulates GSH production. Potentially oxidative damage increases during nutrient starvation which GSH combats.

In bacteria GSH is synthesized in locations where it is needed. In *E. coli* GSH is located in the cytoplasm where it serves to reduce the environment in this compartment [6]. The periplasm of bacterial cells such as *E. coli* is much more oxidized. In fact many proteins in this location are linked by disulfide bonds and consequently GSH is low in this bacterial location. It is thus unexpected that GSH from *E. coli* cells accumulates in the growth media and may serve to protect these bacteria from external oxidative stress before it reaches them [6]. Other bacteria such as cyanobacteria which perform aerobic photosynthesis also synthesize GSH. In this case GSH is located in the cell wall as well as the cytoplasm as ROS can localize to the cell envelope [7].

A number of gram positive organisms produce GSH which include *streptococci*, *listeria*, *lactobacilli*, *clostridium*, and *enterococci*. Gram positive bacteria use differing strategies to produce GSH; some use two separate enzymes which are γ-glutamylcysteine synthetase and GSH synthetase. This is similar to what is seen in *E. coli*. Other bacteria such as *Streptococcus agalactiae*, *Listeria monocytogenes*, *Pasturella multocida* and *Streptococcus thermophilus* contain both functions of the above two enzymes in one [8–11]. In addition some gram positives produce GSH transferases that serve to inactivate toxic compounds [12,13].

As discussed above GSH is synthesized by some bacteria in response to oxidative stress. GSH however is consumed during neutralization of oxidative stress in an oxidized form where two GSH molecules are linked by a disulfide bond. In order to recycle the GSH molecule so that it can be used to combat oxidative damage, the enzyme GSR is induced [14]. This enzyme thus reduces GSSG and is regulated by the transcriptional regulator OxyR [6].

There are a number of bacteria that fail to synthesize GSH but nonetheless can import this molecule from an extracellular location. *Francicella tularensis* (*F. tularensis*) is one of these organisms. In this case *F. tularensis* may use GSH as a nutrient for growth [15]. As *F. tularensis* is a fastidious organism it requires cysteine in growth

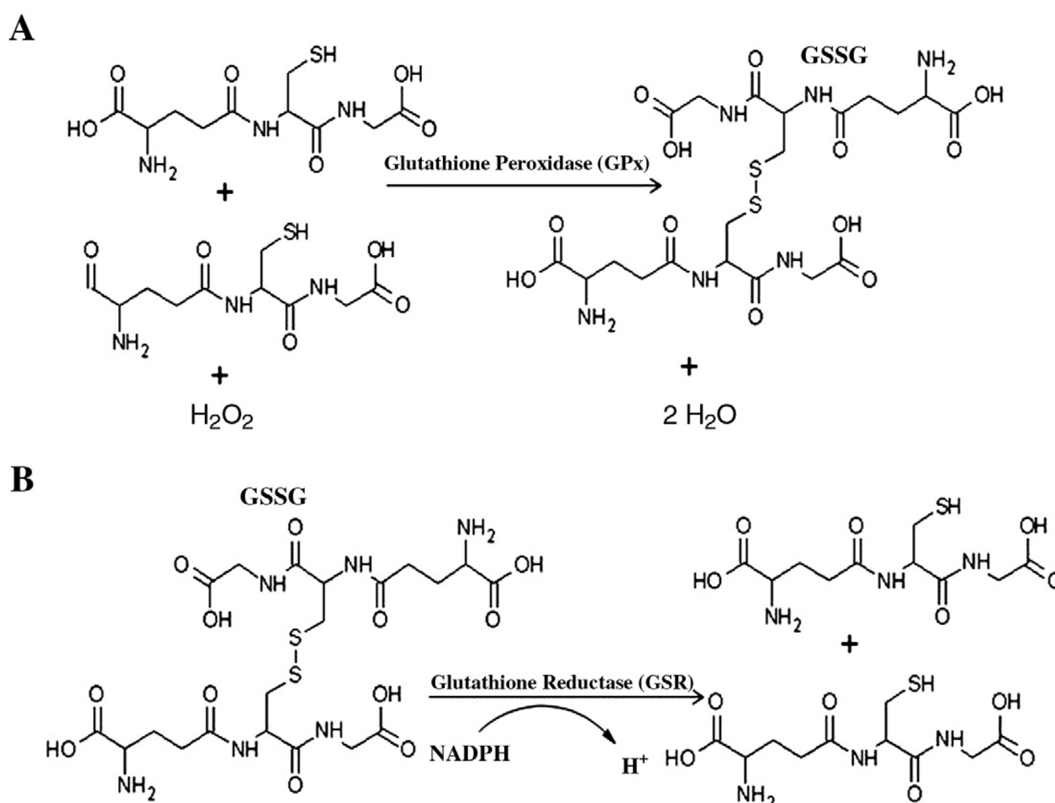

Fig. 2. A: Depicts GPx performing the reduction of hydrogen peroxide to water, while linking two GSH molecules together via a disulfide bridge to form oxidized glutathione (GSSG). B: Utilizing NADPH as a co-factor, GSR performs the reduction of GSSG to GSH, and oxidation of NADPH to $NAD^{+\cdot}$

media and *in vivo F. tularensis* acquires cysteine from the macrophages they infect. As GSH contains cysteine and consequently sulfur, *F. tularensis* imports this molecule using an enzyme, γ-glutamyl transpeptidase (GGT) that cleaves GSH and transports the resultant products into the bacterial cell. In fact GGT is required for growth in a host to manage cysteine starvation. Unlike *F. tularensis*, *Streptococcus mutans*, *Haemophilus influenza* and *Helicobacter pylori* can import GSH directly across the cell wall which can aid these bacteria to resist oxidative damage [16–19]. Mycobacteria can also import GSH. However, in contrast to other bacteria, GSH is actually toxic to these bacilli. Mycobacteria produce another anti-oxidant, mycothiol and the GSH transporter GGT is dispensable for bacterial growth. Deletion of the gene for the transporter from the mycobacterial genome also helps mycobacteria resist toxic effects of GSH [20]. It is not known conclusively why GSH is toxic to mycobacteria, but perhaps it disturbs the redox balance of the bacilli [21]. Thus as macrophages produce GSH as an antioxidant it has the added benefit that it is also toxic to intracellularly replicating mycobacteria [22].

Some bacteria such as *S. thermophiles* and *E. faecalis* upregulate key genes involved in oxidative damage during aerobic growth. A number of agriculturally important bacteria utilize GSH in this manner as well. A bacterium that is used in cheese making, *Lactococcus lactis*, can import GSH to aid this bacterium's resistance to oxidative damage. As oxidative damage increases, the gene coding for the enzyme GSR is upregulated which can then reduce oxidized GSH and recycle this molecule. In conditions where bacteria are growing in the presence of oxygen and are well suspended in growth media, levels of GSR increases indicating oxidative damage increases under these conditions. Studies have shown that various strains of *L. lactis* have variable inductions of GSR which may indicate varying levels of resistance to oxidative damage. Ultimately this characteristic may correlate to strains that have improved ability to act as a starter culture in cheese manufacturing.

GSH can participate in virulence in some pathogens beyond simply providing resistance to oxidative damage. *Treponema denticola* causes dental disease and periodontitis that can result in gingival pockets. *T. denticola* utilizes GSH in growth media to produce hydrogen sulfide (H_2S) which damages host tissues such as gums [23]. Elaboration of this product could encourage pocket formation in the gingiva and thus allow for improved growth of *T. denticola*. Another metabolite of GSH is pyruvate which has been shown to increase bacterial growth and thus improve colonization of dental pockets by these treponemes.

GSH seems to be protective in other stress conditions in addition to oxidative stress. Cold stress and cycles of freezing and thawing can damage *Lactobacillus sanfranciscensis* and GSH is protective under these conditions [24]. Potentially the protection during cold stress is provided by inhibition of peroxidation of membrane fatty acids which in turn act to stabilize membranes and inhibit sensitivity to environmental stresses. In addition heavy metal exposure can lead to damage in *E. coli* which GSH synthesis can inhibit [25]. This seems to be particularly important in mutant *E. coli* that are deficient in heavy metal export systems that normally aid to detoxify bacteria. An herbicide such as atrazine is also capable of inducing GSH s-tranferase in *Bacillus subtilus* and the *E. coli* strain K12. It appears atrazine induces oxidative damage as well [26]. During exposure of cyanobacteria to antibiotic, GSH can increase resistance of the bacteria to the treatment by potentially combating oxidative damage [27]. In the photosynthetic bacterium *Rhodospirillum rubrum*, GSH can increase intracytoplasmic photosynthetic membranes again by decreasing oxidative stress that augments during photosynthesis [28].

Probiotic bacteria such as *lactobacilli* possess strong antioxidant potential. Inflammatory diseases in the gut such as inflammatory bowel disease, Crohn's disease, and ulcerative colitis may be positively modulated by the antioxidant activity of resident intestinal bacteria. Antioxidant potential of lactobacilli from the gut of Indian subjects varied by strain, indicating variability among commensal bacteria

[29]. GSH peroxidase-1 was upregulated in these strains to varying degrees and may aid the *lactobacilli* to resistant oxidative damage and increase resistance in the human host, while potentially benefitting the host through increased antioxidant activity.

3. Tuberculosis

TB is the leading bacterial cause of death worldwide. The causative agent for causing TB is *M. tb* which is responsible for causing 8.8 million new cases of active disease and 1.6 million deaths per year [30]. Much of the burden of disease lies in the developing world, where annual incidence can reach 700 per 100,000 in certain regions [30]. New and unrecognized cases drive the epidemic, with transmission usually occurring before the index case is diagnosed. Multi-drug resistant cases and HIV co-infection further complicate control efforts [31]. Pulmonary TB is the most frequent clinical and transmissible manifestation of active disease. Rapid diagnosis and treatment are critical in the prevention of transmission. Both innate and adaptive immune responses are critical for controlling *M. tb* infection. Our lab has reported a significant decrease in the levels of GSH in peripheral blood mononuclear cells (PBMCs) and red blood cells (RBCs) isolated from individuals with active pulmonary TB, compared to healthy subjects [32]. The percent decrease in the levels of intracellular GSH was more than 70% in PBMC and 30% in RBC, isolated from individuals with active TB. The decreased levels of GSH in individuals with pulmonary TB correlated with increased production of pro-inflammatory cytokines and enhanced growth of *M. tb* [32].

3.1. Innate immune responses against M. tb

Innate immunity, a non-specific immune response provides first line of defense against infections [33] and occurs even prior to the onset of adaptive response (i.e. the production of significant antigen specific B and T cells). Important components of the innate immune system include antimicrobial peptides, proteins, phagocytic cells such as neutrophils and macrophages, as well as non-phagocytic cells such as NK cells. Physical barriers (skin, mucous membranes), chemicals (stomach acid), proteins form barriers that protect against infections [33,34]. Innate immunity is the initial response to infection because its components such as the production of antimicrobial peptides are gene-encoded and are expressed without antigen mediation [34].

Phagocytic cells such as macrophages (Fig. 3) and neutrophils (Fig. 6) phagocytize foreign pathogens such as bacteria by recognizing the Pathogen Associated Molecular Patterns (PAMPs) [33]. The phagocytic pathway allows the macrophages and neutrophils to internalize microbes into compartments where the microbes are brought into contact with ROS, reactive nitrogen intermediates (RNI), antimicrobial peptides, and a generally acidic and hydrolytic environment, which facilitates killing of these internalized pathogens [35]. The first stage step in *M. tb* infection occurs when the alveolar macrophage internalizes the bacilli following inhalation of droplets. Consequently, macrophages in the alveoli are the first cells to become infected by *M. tb* [36]. Macrophages being professional phagocytic cells (Fig. 3) are the first line of defense against microbial invasion [36,37].

3.2. Direct antimycobacterial effects of GSH and control of M. tb infection inside macrophages

Macrophages are the major phagocytic cells involved in controlling *M. tb* infection (Fig. 3). Macrophages acquire the ability to kill intracellular pathogens after exposure to cytokines that are released by antigen-sensitized T-lymphocytes [22,34,36,38]. Activation of macrophages leads to the production of several antimicrobial molecules involved in combating intracellular mycobacterial infection including ROS and RNIs [39–42]. When host cells, such as macrophages, generate ROS and RNI, there will be simultaneous synthesis of GSH for protection against the toxic effects of ROS and RNI [43]. ROS include molecules such as superoxide, hydrogen peroxide (H_2O_2), hydroxyl radicals and singlet oxygen all of which contribute to the control of microbial infections. However, bacteria such as *M. tb* produce catalase that can degrade H_2O_2 [35,36]. RNI are important antimicrobial effector molecules and are more potent compared to ROS in contributing to innate defense against microbial infections. Nitric oxide (NO), an important member of the RNI family has been shown to inhibit the growth of *M. tb* in the murine system [39,44–47]. Although NO is considered as a major effector molecule involved in the control of *M. tb* infection it has a short biological activity as it gets rapidly detoxified to nitrate and nitrite. Both nitrate and nitrite lack antimicrobial effects. However, NO can react with GSH to form *S*-nitrosoglutathione (GSNO) [48]. GSNO, an NO donor, can then release NO, leading to the death of the pathogen [49,50]. The stability of NO is increased when it is in complex with GSH. We have previously shown that 5 mM GSNO has cidal effects on the growth of *M. tb* [51,52]. The sensitivity of *M. tb* to GSNO is due to the bactericidal effects of NO released from the GSNO complex. While RNI have been shown to play an important role in control of *M. tb* infection in the murine system, their role in human disease is not well established. Our group has demonstrated that *M. tb* is also sensitive to GSH [51,52]. We reported that GSH plays an important role in limiting the intracellular growth of *M. bovis* BCG intracellular BCG growth in macrophages derived from inducible nitric oxide synthase (NOS2) knock-out mice and in human monocyte derived macrophages (HMDM) [51]. We have also observed that the virulent laboratory strain of *M. tb*, H37Rv is sensitive to GSH at physiological concentrations (5 mM) when grown *in vitro* [52]. In other words, GSH has direct antimycobacterial activity distinct from its role as a NO carrier [51]. This is an important distinction, since the role of NO in human mycobacterial immunity is uncertain. These results unfold a novel and potentially important innate defense mechanism (Fig. 4) adopted by human macrophages to control *M. tb* infection [60,62]. The antimycobacterial activity of GSH is due to the following reasons:

1) Mycobacteria do not produce GSH. Instead, they have mycothiols for regulating their reduction or oxidation activities [53,54]. Therefore exposure of mycobacteria to high concentrations of GSH may create a redox imbalance in this bacterium containing an alternative thiol for regulating reduction/oxidation activity (i.e., mycothiol) thereby leading to growth inhibition.
2) According to Spallholz; GSH is an evolutionary precursor of antibiotics produced by higher eukaryotes before the emergence of cellular immunity [55]. GSH is structurally similar to the precursor of the antibiotics produced in fungi in the genera *Penicillium* and

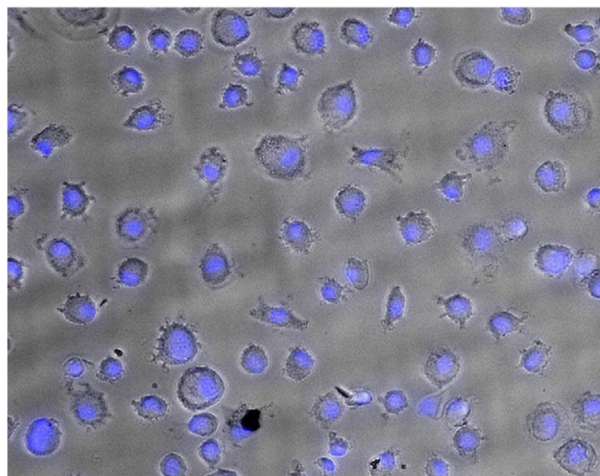

Fig. 3. Illustrates morphology of human monocyte derived macrophages in bright field view (40×) overlaid with fluorescent microscopy image of kidney shaped nuclei stained with 4′, 6-diamidino-2-phenylindole (DAPI-blue color).

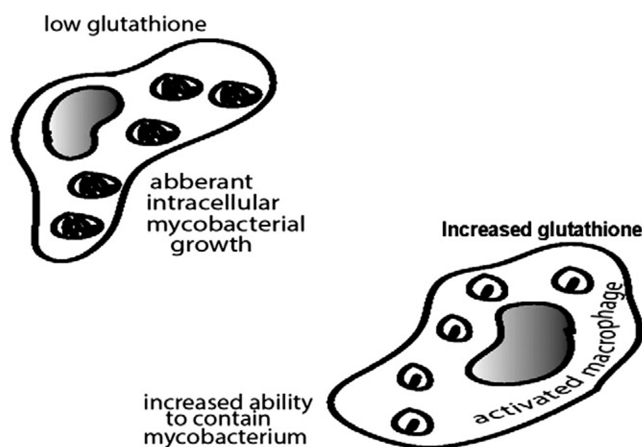

Fig. 4. Demonstrates the direct antimycobacterial effects of GSH in limiting the intracellular growth of *M. tb* in macrophages by showing the reduction in the number of bacteria in the second image after incubation with GSH.

Cephalasporium. Its potential conversion to the penicillin-like derivative glutacillin, a β-lactam form of GSH, raises the intriguing question of whether GSH was once a universal penem-like precursor of antibiotics in cells of many life forms [55]. The mycobacterial cell wall may possess some intrinsic sensitivity to this structure.

3) Importantly, GSH is a precursor for GSNO, and GSNO may represent one of the most important active forms of NO as an antimicrobial agent [44,49,56,57]. GSH has further antimycobacterial effects, helping macrophages to control intracellular *M. tb* infection by modulating macrophage responses and altering the profile of cytokines secreted by infected macrophages [58].

3.3. GSH-enhanced NK cells and control of M. tb infection inside macrophages (indirect effects of GSH)

Like macrophages, NK cells also play an important role in the innate immune defense against *M. tb* infection [59–65]. NK cells are defined phenotypically as large granular lymphocytes that express both CD16 and CD56, and functionally as cells that mediate non-MHC-restricted cytotoxicity against a variety of target cells [66]. NK cells express cell surface ligands that are capable of inducing apoptosis [63–73]. Apoptosis of infected cells may lead to killing of intracellular pathogen.

Our studies have shown that the cytolytic activities of NK cells are severely impaired under low GSH conditions [63,64]. We have also observed that treatment of NK cells with IL-2 + IL-12 + N-acetyl cysteine (NAC) causes a significant increase in NK cell cytolytic activity [63,64]. There is substantial evidence from our laboratory indicating that NK cells and macrophages work in conjunction to combat intracellular *M. tb* infection, and GSH has been shown to enhance the functions of NK cells to control *M. tb* infection inside macrophages [63,64]. Our lab has also demonstrated that treatment of NK cells with NAC in combination with IL-2 and IL-12 results in control of *M. tb* infection (Fig. 5) and the growth inhibitory effects correlated with increased expression of FasL and CD40L on the cell surface of NK cells. Neutralization of FasL and CD40L on the NK cell surface resulted in abrogation in the growth inhibition and several-fold increase in growth of *M. tb*, indicating a novel pathway by which NK cells control the growth of *M. tb* inside human monocytes (Fig. 5).

3.4. GSH mediated control of M. tb infection in neutrophils

GSH also seems to have an enhancing effect on neutrophils, which are also phagocytic cells that are a part of the innate immune system (Fig. 6). Based on our most recent unpublished studies, treatment of neutrophils with a liposomal formulation of GSH [LGSH (10 μM)] inhibits *M. tb* growth within neutrophils, while other treatment with a GSH synthesis inhibitor, BSO resulted in enhanced mycobacterial growth in neutrophils at 24 h post-treatment.

3.5. GSH and the adaptive immune cells

Adaptive immune cells work in close conjunction with innate immune cells to combat pathogens that attack the human body. Much of the interactions between adaptive and innate immune cells are in the form of peptide antigen presentation in the context of major histocompatability complex. GSH is noted to be integral in the processing of microbial peptide, through the exogenous route of antigen presenting cells (APCs) [74–76]. The reduction of disulfide bridges inherent in the protein degradation process in lysosomes is achieved by GSH's oxidized cystine and is one of the first steps of peptide processing by acting as an electron sink [77]. In order to mount a proper immune response naïve adaptive immune cells must undergo these interactions as well as specific differentiation through precise and distinct signaling [78]. GSH has been shown to alter cytokine expression specifically by enhancement through NAC [32,58,64,79] and γ-glutamylcysteine synthase [80]. It is clear that the deficiency of GSH in, both genetic (oxoprolinuria) and acquired (AIDS infected and septic patients), individuals have greater difficulty clearing microbial infections and central nervous system dysfunction [74,81]. To assume the correlation between the absence of GSH and difficulty in clearing microbial infections one must understand the specific roles of how GSH functions in adaptive immune cells. It is the specific goal of this section to outline the roles of GSH functions in these adaptive immune cells.

3.6. T-cells and GSH in the context of M. tb

The mechanism by which *M. tb* proliferates poses a difficult problem for the immune system to efficiently mount an adequate protective response. The shear lack of diagnostic biomarkers for disease progression indicates the severity of the elusiveness of these bacilli, specifically because well maintained clinical control yields the same results as disease onset and latency [82]. The initial site of infection takes place in the alveoli in the lungs, where they are phagocytosed by APCs such as macrophages [79,82]. Once phagocytosed the macrophages undergo loss of control of phago-lysosomal fusion mechanisms such as sequestering of the proper coat proteins tryptophan and aspartate (TACO proteins), anionic trehalose glycolipids known as sulfatides, and ammonia production [82,83]. Other mechanisms that increase *M. tb*'s survival include acidification deficiency in phagosomes (proton ATPase pump inhibition) [83], the up regulation and expression of virulence glycine-rich proteins [83] and finally the arsenal of genes that give protective effects from ROS (catalase and hydroperoxidase) [83]. By preventing the fusion, *M. tb* carries a high survival rate proof to its clinical latency and infectious rates, (evident by 8.8 million people being infected and 1.4 million deaths in 2010 alone [WHO]). Once the onset of replication takes place the formation of granulomas composed of macrophages and T cells in the lungs can be visualized with a simple chest X-ray. Levels of cytokines begin to build up and the cellular employees of adaptive immune system initiate their offense.

The significance of free radicals in signaling mechanisms and redox regulation have been implicated as a key factor in T-cell homeostasis [84]. The literature indicates that many of the different cellular functions are under the influenced by the redox environments both intra [85] and extra [32,86] cellularly [84]. T-cells have been shown to require ROS for both proliferation (NF-κB, erk2, and AP-1) and apoptosis (*Bcl-2*, *FasL* transcription) [87]. The effects of GSH in many immunocompromised individuals, particularly to those who are immunocompromised such as HIV patients, has shown great advance in managing *M. tb* [32]. Control in these individuals is achieved by supplying GSH to delicately balance the optimal concentrations of ROS to both proliferate and elicit

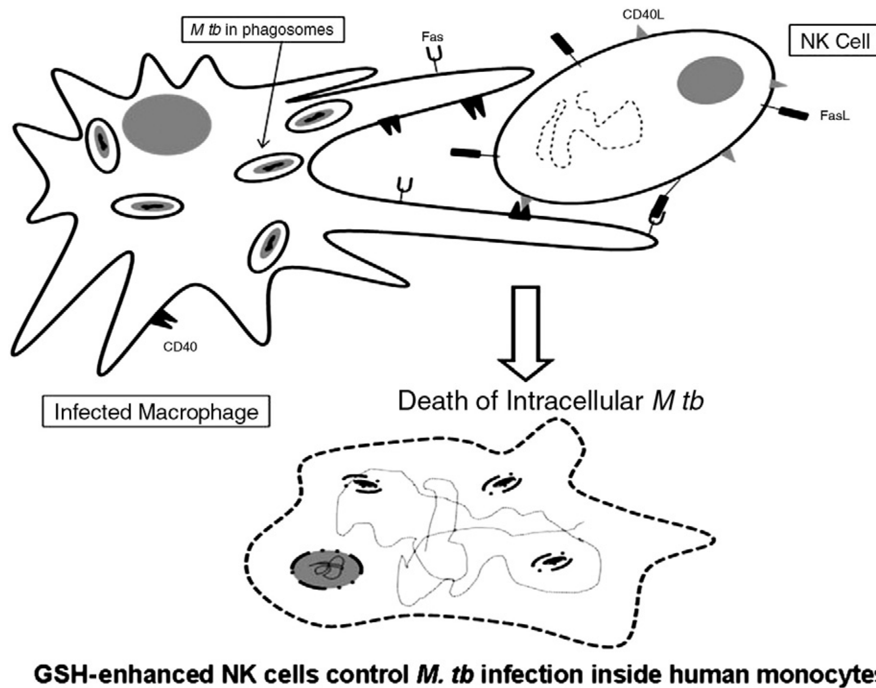

Fig. 5. Demonstrates that treatment of NK cells with NAC in combination with IL-2 and IL-12 results in decreased survival of *M. tb* inside human monocytes and that this inhibition in the growth of *M. tb* is due to increased expressions of FasL and CD40L on the cell surface of NK cells.

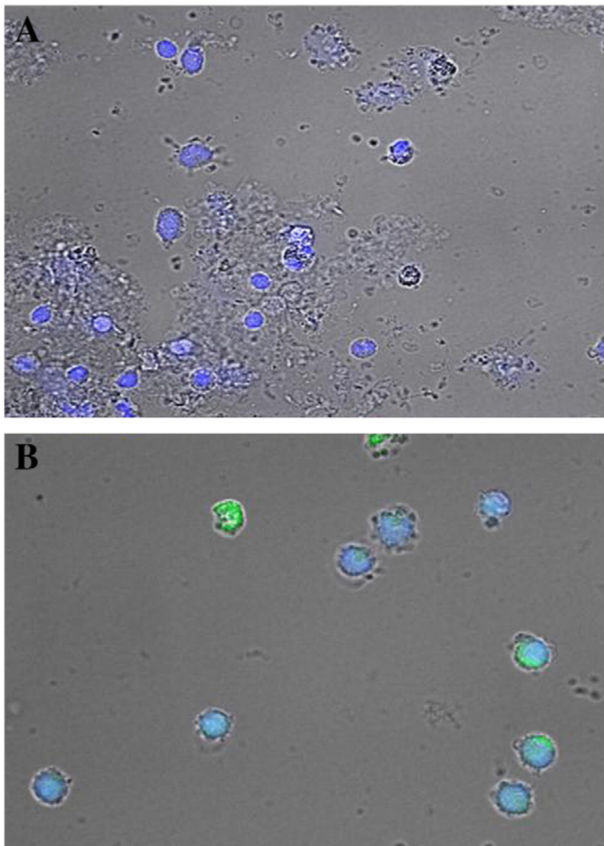

Fig. 6. Microscopic images of neutrophils. A: Bright field view (40X) overlaid with fluorescent microscopy image of nuclear staining with DAPI (blue staining) showing the shape and morphology of human neutrophils. B: Green FITC staining of intracellular *M. tb* inside human neutrophils at 40× magnification.

the correct immune response through ideal interleukin concentrations [79]. For instance, levels of interleukins in the proper concentrations in *M. tb* infected neutrophils can evoke apoptosis thereby properly disposing of bacilli, while elevated concentrations of interleukins can cause a necrotic effect resulting in *M. tb* survival [88,68]. It is still too early to determine the optimal levels of interleukin proteins for T-cells and further work remains to be completed in order to identify the role of T-cells in *M. tb* patients. Our studies signify the importance of GSH as an antimycobacterial agent that can potentiate control of *M. tb* infection inside monocytes and macrophages. Our studies also highlight the immune enhancing effects of GSH in improving the functions of NK and T cells (Figs. 5 and 8) to control *M. tb* infection inside monocytes.

3.7. TH1/TH2

T-helper cells previously unexposed to antigen are deemed naïve T-helper cells. Naïve T-helper cells differentiate to effector cells after TCR/CD3 and MHC II co-stimulatory signaling as well as cytokine microenvironment [89,90]. Of the five possible routes a T-helper cell can differentiate into (T_H1, T_H2, T_H3, T_H17, T_{FH}), in this section we will closely examine the effects of GSH on “cellular immunity” and “humoral immunity” pathways or T_H1 and T_H2 pathways respectively. These cells have no cytotoxic activity and are not responsible for any direct killing but are still deemed necessary to mount a proper and robust immune response [91]. In addition, they assist and activate other adaptive and innate immune cells by releasing protein cytokines for an appropriate response serving as supervisors and directors [89].

Depending on the nature of cytokines in the environment and APC peptide direct contact, naïve T-helper cells will mature into two major lineages. These different lineages each require different profiles of cytokines. T_H1 for example requires IL-12 and IFN-γ [90], these cytokines act as both autocrine and paracrine function influencing the surrounding cells in the microenvironment. The IFN-γ released by T_H1 cells blocks the proliferation of T_H2 and T_H17 cells, thereby mounting the proper response to correlating pathogens [90]. In particular, the release of cytokine profiles in T-cells is altered by the levels of free radicals seen in the intracellular environment [84].

GSH has been reported to alter the release of cytokines to a pro-T_H1 response [92]. Researchers found that *in vitro* treatment of whole blood (isolated from individuals with HIV infection and infected *in vitro* with *M. tb*) with NAC resulted in increased production of IFN-γ thereby strengthening a T_H1 response allowing for better control of *M. tb* [92,93]. NAC is a metabolite of the sulfur-containing amino acid, cysteine. The results of this study imply that intracellular levels of GSH is key for mounting a T_H1 immune-response resulting in favorable cytokine production leading to control of *M. tb* infection. The proposed mechanism for which the altered cytokines profiles are due to GSH depleting ROS, this depletion of ROS leads to NF- κB to stay sequestered by I κB in the cytoplasm. NF-κB has been suggested as a transcription factor for cytokines IL-1, IL-6, and TNF-α [84,93,94].

Numerous other studies have shown that *M. tb* control favors a higher T_H1 activity and lower T_H2 shift [90,92,93,95,96]. A Study on 23 Mexican individuals with *M. tb* employed descriptive flow cytometry to statistically show that CD28 co-stimulatory molecule was greatly down-regulated on both CD4+ and CD8+ cells ($P<.001$). Research shows that CD28 is a molecule essential in T_H1 stimulation and proliferation. Patients had higher susceptibility to recurrent or relapsing episodes of *M. tb* (47.8%) [95]. The recent focus of the CD28/B7 co-stimulation on knock-out mice has also implicated the importance of a T_H1 response when mounting an attack against *M. tb*. Researchers aerosolized B7DKO mice with *M. tb* Erdman and were found unable to fight chronic tuberculosis infection due to the lack of a T_H1 response ultimately resulting in necrosis of the lungs due to granulomas [84,96].

3.8. Dendritic cells (DCs)

The most potent APC that has been shown to induce T-cell activation and differentiation to effector cells is the DCs [97]. DCs (Fig. 7) are regarded as the bridge that intertwines the innate and adaptive immune systems [98]. Recent findings show that these sentinels excrete cysteine (limiting reagent for GSH synthesis) and are responsible providing a reducing localized microenvironment for naïve CD4+ T-cells to differentiate [84,86,99,100]. It is noted that cysteine is found in the lowest concentration of all amino acids circulating in the blood [84].

NAC has been shown to inhibit NF-κB in both baseline and LPS-challenged DCs [94]. The loss in redox signaling is a result of a NAC increasing the intracellular GSH levels and reducing extracellular levels of ROS. These ROS serve as pro-inflammatory modulators. Pro-inflammatory cytokine storms have their benefits such as localization and recruitment of immune cell, but they can also cause tissue damage and necrosis of immune cells in high concentrations [101,102,22]. We must also remember that the proper cytokines are important to elicit the proper immune response, as in the case of an intracellular pathogen such as viruses and mycobacterium T_H1 cytokine profiles are favored for better control.

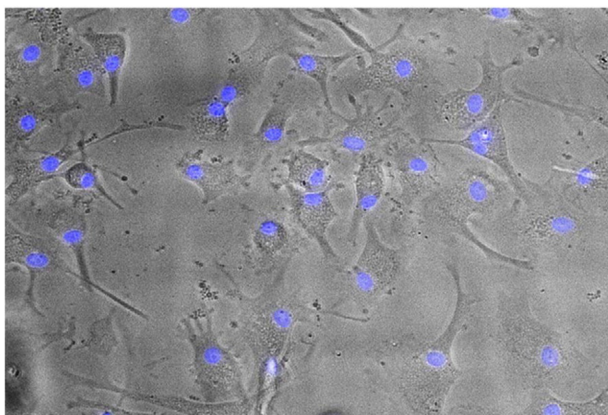

Fig. 7. Fig. 7 shows dendritic cells (also considered as professional antigen presenting cells) with extensions called dendrides that aid in the trapping of antigens. Image shown is a bright field view of human dendritic cells (40×) overlaid with fluorescent microscopy image of DAPI-stained nucleus.

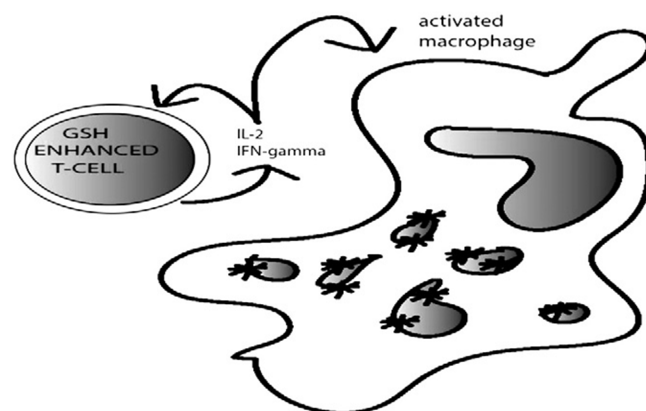

Fig. 8. Demonstrates the important role of GSH in enhancing the functions of T lymphocytes to limit the intracellular growth of *M. tb* inside macrophages, by producing macrophage activating cytokines such as IFN-γ.

More evidence suggests that depletion of GSH in murine models results in a decrease in IL-12 production in DCs [103]. The loss of IL-12 is significant due to its requirement in the maturation process of DCs. This cascade of events of GSH depletion by diethyl maleate correlates to a low concentration in IFN-γ found on murine skin after administration of known delayed type hypersensitivity adjuvants (contact-sensitizing antigens, oxazolone, and 2,4-dinitro-1-fluorobenzene). The results of the study suggest that the loss of T_H1 response due to aging in mouse models can be reversed by replenishing GSH with NAC [103].

GSH and DC maturation is also implicated by another group that studied the effects of cysteine/glutamate antiporter found on the membrane of DCs using radiolabeled amino acids glutamate and cysteine [78]. Researchers found that inhibition of the antiporter with L-homocysteic acid (LHC) resulted in reduction of intracellular GSH and implicated that GSH may play an important role in maturation of DCs.

IL-27 has been recently anointed as a key cytokine released by DCs responsible for the differentiation of naïve T-cells. Researchers directly related the production of IL-27 to the redox status of DCs due to intracellular levels of GSH [104]. Kamide et al. accomplished this by enhancing DCs with GSH-OEt and then challenged them with LPS.

3.9. Regulatory T cells (T-regs)

T-regs sub-sets are regarded as the protectors of self against self. Rogue CD4+ cells that mature to recognize self-antigen as foreign are kept in-check by these regulatory cells through suppression mechanisms, ultimately safeguarding against autoimmune diseases. These cells have been identified to block the synthesis of GSH by inhibiting transcription of the limiting enzyme γ-glutamylcysteinyl ligase thereby causing a depletion of GSH availability [80]. The result of low GSH due to low concentrations of extracellular available cysteine for DCs in particular to utilize, is due to T-reg immunoregulation. The effects of increased GSH availability with respect to T-regs remain a question; however that question that is currently being put to the test, and studied.

4. HIV and AIDS

According to the World Health Organization, a staggering 34 million people in the world today are infected with HIV. Since the first reported case in 1981, which started the epidemic, 25 million individuals have lost their lives to AIDS related causes [105]. HIV infection is associated with a myriad of opportunistic infections which ultimately result in poor patient survival. Some of these infections include *Kaposi sarcoma*,

Pneumocystic pneumonia, *Herpes virus* and *M. tb* [106]. These infections flourish due to the compromised immune system which follows the depletion of the CD4+ T cells [107]. Along with the depletion of CD4+ T cells, other immune cell types have been demonstrated to be malfunctioning in cases of HIV infection, these cells include macrophages and NK cells [108].

4.1. HIV and increased susceptibility to M. tb infection

Majority of the 34 million people living with HIV today live in sub-Saharan Africa, 22.9 million to be exact, incidentally this sub Saharan region is a region where *M. tb* is endemic [109]. One of the opportunistic infection that is detrimental to HIV patients once the disease progresses to AIDS is *M. tb* infection. *M. tb* remains one of the front runners in causing opportunistic infections in individuals with a compromised immune system, and worldwide it remains as a malicious pathogen. In recent years there has been a significant increase in the incidence of *M. tb* infection due to the emergence drug resistant strains of *M. tb* and activation of latent *M. tb* infection in increased numbers of highly susceptible immunocompromised individuals arising from the AIDS pandemic. In addition, it is believed that in developing countries, as many as 80% of individuals with AIDS are at risk of developing TB [110,111].

4.2. Decreased GSH in HIV infection and increased susceptibility to M. tb

Our studies have shown that HIV infected individuals have deficiencies of intracellular GSH in RBCs as well as PBMCs, which include T cells, NK cells, and monocytes [58,79,93]. We also recently reported that the levels of intracellular GSH in macrophages from HIV+ individuals are compromised [57]. Furthermore, we examined the causes for decreased GSH in individuals with HIV infection. We observed lower levels of intracellular GSH in macrophages from individuals with HIV compared to healthy subjects. Further, the GSH composition found in macrophages from HIV+ subjects heavily favors oxidized glutathione (GSSG) which lacks antioxidant activity, over free GSH which is responsible for GSH's antioxidant activity. This decrease correlated with an increase in the growth of *M. tb* in macrophages from HIV+ individuals. In addition, we observed increased levels of free radicals, pro-inflammatory cytokines (IL-1, IL-17 and TNF-α) and transforming growth factor-β (TGF-β) in plasma samples derived from HIV+ individuals compared to healthy subjects [57]. We observed decreased expression of the genes coding for enzymes responsible for de novo synthesis of GSH in macrophages derived from HIV+ subjects using quantitative PCR (qPCR). Our results indicate that overproduction of pro-inflammatory cytokines in HIV+ individuals lead to increased production of free radicals [57]. This combined with the decreased expression of GSH synthesis enzymes leads to a depletion of free GSH and may lead in part to the loss of immune function observed in HIV patients [57]. We hypothesize that supplementing GSH will result in a restoration of intracellular GSH and a corresponding restoration of macrophage function. We tested our hypothesis by performing *in vitro* studies using human monocyte derived macrophages isolated from healthy individuals and individuals with HIV infection. Our results indicate that restoring the levels of GSH reverts the loss of innate immune functions observed in macrophages derived from HIV+ individuals. Taken together, the data demonstrates a pattern of chronic inflammation brought on by HIV infection which depletes reduced GSH, and impairs the intracellular killing of *M. tb* in macrophages [57]. By supplementing reduced GSH we were able to mitigate the production of ROS and improve the ability of macrophages to kill *M. tb* intracellularly. Supplementation of GSH also corresponded with increased production of GCLC and decreased production of GSR, further indicators that conditions of oxidative stress are reduced through the administration GSH supplements. This data makes a compelling argument for the efficacy of GSH supplements in addition to the ART regimen prescribed to HIV+ patients in improving immune function. We recently reported that T lymphocytes that are derived from HIV infected individuals are deficient in GSH, and that this deficiency correlates with decreased levels of T_H1 cytokines (IL-2, IL-12 and IFN-γ) and enhanced growth of *M. tb* inside human macrophages [78]. We also observed that the levels of GSH are decreased significantly in NK cells derived from individuals with HIV infection compared to healthy subjects, and this decrease correlated with a several-fold increase in the growth of *M. tb* inside monocytes [62]. Therefore, GSH deficiency in HIV greatly impairs the innate and adaptive immune responses leading to increased susceptibility to *M. tb* infection.

4.3. GSH and anti-inflammatory effects

In vitro infection of whole blood cultures derived from HIV positive individuals with *M. tb* resulted in an increased production of pro-inflammatory cytokines such as IL-1, TNF-α, IL-6. However, levels of the pro-inflammatory cytokines (IL-1, TNF-α, and IL-6) were reduced when the whole blood cultures were treated with the GSH precursor, NAC [93].

Increased levels of these pro-inflammatory cytokines may be detrimental to the host because it increases the risk of fever, cachexia, hemorrhagic necrosis, and lethal shock [112–114]. Furthermore, elevated levels of pro-inflammatory cytokines such as IL-6 can be a disadvantage to the host because it can interfere with macrophage activity. Researchers found that macrophages infected with *M. bovis* released an abundant amount of IL-6 which in turn inhibited the macrophage's ability to mediate the proliferation of CD4 T-cell hybridomas [37].

The mechanisms by which pro-inflammatory cytokines decrease intracellular GSH may be in response to increased levels of free radicals. A study found that increases in pro-inflammatory cytokines resulted in an increase of free radicals which is targeted by free GSH in host cells. In HIV patients where there is excessive production of pro-inflammatory cytokines, there is a decrease in GSH because the antioxidant is being depleted as it is targeting the free radicals. Furthermore, elevated levels of IL-1 may also contribute to the depletion of intracellular GSH. It is believed that IL-1 facilitates the depletion of intracellular cysteine thus slowing GSH production and decreasing levels of GSH [58].

Our studies in human subjects showed that patients infected with the HIV have lower levels of GSH in their macrophages, NK and T cells compared to individuals [57,62,78]. Others have reported low levels of GSH in epithelial lining fluid (ELF) in individuals infected with HIV. Individuals with HIV infection are at greater risk of developing TB due to reactivation of latent *M. tb* infection [116–119].

GSH contributes to the control of intracellular *M. tb* infection by several different mechanisms. It has direct antimycobacterial effects, can enhance the functions of NK and T cells, acts as an antioxidant, acts as a carrier molecule for NO, and reduces the levels of pro-inflammatory cytokines (IL-1, TNF-α, and IL-6) [22,43,48,51,115,120]. Therefore, GSH plays a vital role in directly controlling mycobacterial infection as well as improving host defenses such as improving the immune cells functions and restoring macrophage function by reducing IL-6 [37,93]. The effects of GSH were evident when immune cells from HIV+ subjects were treated with a GSH-enhancing agent (NAC/L-GSH) was found to improve the control of intracellular *M. tb* infection. [93].

5. GSH and cystic fibrosis (CF)

CF is characterized by several pathological processes potentially capable of damaging the respiratory epithelium. First, there is a chronic accumulation of excessive numbers of activated inflammatory cells. These cells, as part of their inflammatory armamentarium, release exaggerated levels of oxidants. In addition, CF ELF is deficient in GSH, one of the key components of the normal antioxidant defenses of the respiratory epithelium [121]. Although the reasons for the low levels of ELF GSH are not completely understood, the significance of this deficiency is profound. Because GSH is a potent antioxidant capable of scavenging

a variety of oxidant molecules, deficiency of GSH places the protein and lipid moieties of the respiratory epithelial cells, as well as extracellular molecules such as α_1-antitrypsin (α_1-AT), at increased risk for oxidative damage [121]. When oxidized by exogenously or endogenously produced oxidants, α_1-AT becomes ineffective as an inhibitor of neutrophil elastase (NE), thus leaving the lung vulnerable to proteolytic degradation by NE. Consequently, the exaggerated burden of oxidants on the respiratory epithelial surface in CF, along with a deficiency in one of the key antioxidant protective molecules, leaves the CF respiratory epithelium vulnerable to oxidative damage and NE-mediated proteolytic injury. These complex, interrelated pathological processes combine to play a central role in the progressive derangements that occur in the lung in CF [121].

Concomitantly, evidence suggests that an increased oxidant burden actually promotes *Pseudomonas* virulence by inhibiting mucociliary clearance of the organism and allowing for unopposed NE activity due to oxidant-induced antiprotease inhibition (Table 2). In addition to its direct antioxidant role, GSH may act to preserve antiprotease activity in these conditions, as suggested by several *in vitro* cell-free studies. GSH inhibited myeloperoxidase-mediated inactivation of α_1-AT. GSH in combination with GSH peroxidase inhibited loss of lipid peroxidation-induced α_1-AT activity. Catalase-suppressible inhibition of α_1-AT by gas-phase cigarette smoke was also reduced by GSH [121]. In this context, investigators have evaluated the feasibility of aerosol delivery of GSH to the lower respiratory tract in CF to augment the antioxidant barrier of the respiratory epithelium [121]. The findings indicate that aerosol administration of GSH to CF patients effectively increases ELF GSH levels. Moreover, GSSG levels were also increased, suggesting that the delivered GSH was utilized within the lung as an antioxidant. Finally, as indicated by the decrease in O_2^- release by inflammatory cells after GSH aerosol delivery, GSH acts at the level of the inflammatory cell to decrease the oxidant burden on the epithelial surface [121]. Thus GSH aerosol administration is an effective method of augmenting the antioxidant protective barrier of the respiratory epithelium in CF [121,122].

The effects of GSH aerosol therapy appear to be lung specific; i.e., plasma levels of GSH and GSSG were not altered as a result of therapy. In addition, GSH aerosol therapy in CF is safe; no adverse clinical effects were noted in any of the patients. The lack of development of infectious symptoms or signs also argues that reduction of the oxidant burden in CF is not associated with inhibition of CF host defense against microorganisms, a potentially serious problem in the chronically colonized milieu in the CF lower respiratory tract. This is consistent with the finding that, in vitro, extracellular GSH concentrations of up to 300 μM do not inhibit bactericidal or phagocytic ability of neutrophils [121,122]. Moreover, because deficiency of GSH has been associated with abnormal phagocytic cell function and, *in vitro*, increased extracellular GSH prevents exogenous oxidant-induced intracellular GSH depletion and decreased phagocytic capacity by neutrophils, augmentation of ELF GSH levels by aerosol therapy would be expected to improve antibacterial function on the respiratory epithelial surface in CF [121,122].

6. GSH and diabetes

Similar to HIV+ individuals, individuals with Type 2 diabetes mellitus (DM) have decreased levels of GSH and increased susceptibility to infections including *M. tb* infection. DM has been described as a global epidemic. It is estimated that the number of people diagnosed with diabetes is expected to grow from 171 million in 2000 to 366–440 million by 2030 [123]. Uncontrolled diabetes leads to significant morbidity and mortality due to its effects on the microvasculature causing retinopathy, nephropathy and neuropathy and the macrovasculature leading to cardiovascular, cerebrovascular and, peripheral vascular damage. Besides being associated with these major complications, prevalence of infections is also much more common in individuals with DM [124]. There is considerable evidence that hyperglycemia results in the generation of ROS ultimately leading to increased oxidative stress in a variety of tissues. Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and macrovascular [125].

The metabolic abnormalities of DM cause mitochondrial superoxide production. This increased superoxide production causes the activation of 5 major pathways involved in the pathogenesis of complications: polyol pathway flux, increased formation of AGEs (advanced glycation end products), increased expression of the receptor for AGEs (RAGE) and its activating ligands, activation of protein kinase C isoforms, and over activity of the hexosamine pathway. Several lines of evidence indicate that all 5 mechanisms are activated by a single upstream event: mitochondrial overproduction of ROS [125]. Mitochondria, the principal energy-generating organelles in the cell, are required for initiation of hyperglycemia-induced superoxide production, which can, in turn, activate a number of other superoxide production pathways that may amplify the original damaging effect of hyperglycemia. Increased free fatty acid oxidation in mitochondria also produces ROS. Superoxide is the initial oxygen free radical that is formed by the mitochondria which is then converted to other more damaging molecules. Using an antioxidant such as GSH to reduce the amounts of oxygen free radicals could in turn reduce the damage.

6.1. Increased polyol pathway flux

Glucose is converted to sorbitol by the enzyme aldose reductase (AR) [125]. AR activity depletes its co-factor NADPH, which is also required for GSR to regenerate GSH. Under hyperglycemic condition, as much as 30% of the glucose is channeled into the polyol pathway [125] causing a substantial depletion of NADPH and consequently a significant decrease in the GSH level. Thus, during hyperglycemia, AR activity diminishes the cellular GSH-antioxidant capacity.

6.2. Increased Intracellular AGE formation

Intracellular production of AGE precursors can damage cells by modifying intracellular protein structure as well as inducing extracellular matrix component damage. Plasma proteins modified by AGE bind to RAGEs on cells such as macrophages, and vascular endothelial and smooth muscle cells. This RAGE binding in turn activates the pleiotropic transcription factor nuclear factor (NF)-κB [126].

6.3. Increased protein kinase C (PKC) activation

Persistent and excessive activation of several PKC isoforms operates as a third common pathway mediating tissue injury induced by DM-induced ROS [127]. This results primarily from enhanced *de novo* synthesis of diacylglycerol (DAG) from glucose via triose phosphate, whose availability is increased because ROS inhibits activity of the glycolytic enzyme GAPDH (glyceraldehyde-3 phosphate dehydrogenase). Activation of PKC may contribute to the accumulation of microvasculature matrix protein by inducing expression of transforming growth factorβ_1 (TGF β_1), fibronectin and type IV collagen [128–130].

6.4. Increased hexosamine pathway flux

In this pathway, fructose-6-phosphate is diverted from glycolysis to provide substrate for the rate-limiting enzyme of the hexosamine pathway and this increased flux increases the gene transcription of key genes such as TGF β_1, TGF α and PAI-1(plasminogen activator inhibitor-1) in endothelial cells [131–134].

6.5. DM and infection

It is widely accepted that diabetics have an increased propensity to develop infections. These can range in severity superficial skin

infections, urinary tract infections, bacteremia and necrotizing skin and soft tissue infections. Patients with diabetes, particularly those who inject insulin daily, often have asymptomatic nasal and skin colonization with *S. aureus*. Furthermore, according to an analysis of data from the National Health and Nutrition Examination Survey (NHANES) collected between 2001 and 2002, diabetic patients who are colonized with *S. aureus* are more likely to have a methicillin-resistant *S. aureus* isolate than a susceptible one (odds ratio 2.6; 95% CI, 1.1–6.1) [135]. An ex vivo comparison study of production of T_H1 cytokines showed that nonspecific IFN-γ levels were significantly reduced in people with diabetes compared to controls without diabetes [136]. Another study indicated a dose–response relationship; levels of IFN-γ were negatively correlated with levels of HbA1c (a measure of serum glucose levels over time in humans) [137]. Furthermore, neutrophils from people with DM have reduced chemotaxis and oxidative killing potential than those of nondiabetic controls [138], and leukocyte bactericidal activity is reduced in people with diabetes, especially those with poor glucose control [124]. Taken together, these studies strongly support the hypothesis that DM directly impairs the innate and adaptive immune responses necessary to counter the proliferation of TB. It remains to be determined what leads to this impairment in the immune response in DM.

6.6. DM and TB

A number of recent literature reviews, which have included systematic reviews and meta-analyses of previous studies, have shown that DM patients have a significantly increased risk of developing active TB, which is two to three times higher than in persons without DM [139–142]. In a meta-analysis of cohort studies Jeon et al. showed that individuals with DM was associated with an increased risk of TB (RR 3.11, 95%CI 2.27 to 4.26), though there was a moderate degree of heterogeneity detected. The high degree of heterogeneity among case–control and other observational study designs precluded statistical pooling, but the qualitative summary of individual study results showed that in all studies, there was an increased risk of TB. Subgroup analysis within this meta-analysis showed that regardless of background incidence of TB or underlying medical conditions, there is an increased risk of TB in patients with DM.

A well-conducted systematic review and meta-analysis [143] evaluated the effect of diabetes on treatment outcomes on patients with TB. This systematic review synthesized studies which evaluated the association between presence of DM and the risk of sputum culture conversion, treatment failure, death, or relapse. DM increases the risk of failure and death combined {pooled RR = 1.69 (95% CI, 1.36 to 2.12)}, death {pooled RR = 1.89 (95% CI, 1.52 to 2.36)}, and relapse {pooled RR = 3.89 (95% CI, 2.43 to 6.23)} among patients with TB. Due to a high degree of heterogeneity, studies evaluating sputum culture conversion were not pooled. However, most studies did trend toward an increased risk of remaining sputum culture positive in TB patients with DM compared to TB patients without DM [143].

Thus, DM increases the general risk of infection with TB, but the precise mechanisms by which DM predisposes to TB are still not clear. DM does not reduce CD4 + T lymphocyte populations, but rather decreases the synthesis of GSH, impairs the function and activation of macrophages, monocytes and lymphocytes which play a pivotal role in combating the TB pathogen [139,140,144,145]. DM patients with poorer glycemic control appear to be at higher risk for TB [146,147] demonstrating a dose–response relationship between the degree and duration of hyperglycemia and vulnerability to TB (Table 2).

6.7. Diabetic oxidative stress and TB

It has been suggested that oxidative stress in DM may be one of the mechanisms leading to increased susceptibility to infections. Diabetics have decreased GSH levels [125]. Tan et al. (2012) recently have shown that depletion of GSH in a mouse model of melioidosis contributes to decreased IL-12 production, increased disease susceptibility, and poorer disease outcome. The authors extrapolate this *ex vivo* data and suggest that GSH deficiency in PBMCs from diabetics may also account for the impaired ability to control *M. tb* infection due to a defect in IL-12 production [148].

7. GSH and other viral infections

Many of the mechanisms of viral pathophysiology involve an imbalance of the redox balance of the cell in favor of oxidation stress with a resulting dysregulation of GSH [149]. GSH in the reduced form is the major intracellular redox buffer and plays a critical role in protecting cells against damage from oxidants [150]. *In vitro* cell culture studies of viral infection show that oxidative stress and a decrease in intracellular GSH occurs in the host cells after infection with viruses including, HIV, hepatitis C [151], herpes simplex type 1 [152], Sendai (parainfluenza) virus [153], rhinovirus [154] and influenza virus [155].

A mouse model of influenza A virus infection confirms the loss of reduced GSH *in vivo* [156]. The study also presents the observation that the virus alone has limited cytopathic effect on the epithelial cells lining the respiratory tract and does not explain the complication of pneumonia resulting from infection [156]. The findings suggest that the tissue damage may be related to the host inflammatory response to the virus and not directly from the virus. It appears that the effector mechanisms involved in the clearance of pathogens such as the ROS generated by phagocytes participate in the disease [157,158]. ROS can also contribute to the damage seen in the lungs during viral infection by oxidizing lipids and damaging membranes, proteins and nucleic acid [159] or inactivating critical enzymes [160]. Bronchoalveolar lavage (BAL) fluid from mice infected with influenza virus show decreased total GSH, increased GSSG, and an increased level of malondialdehyde, which is an indicator of lipid peroxidation [161].

7.1. Cellular loss of GSH after viral infection is a two-step process

Detailed evaluation of cellular loss of GSH associated with Sendai (parainfluenza virus), an RNA virus infection shows that after viral infection, GSH is lost in a two-step fashion [153]. The first step of loss occurs with the initial interaction between the virus and the cell membrane. Madin–Darby canine kidney (MDCK) cells infected with Sendai virus show a time-dependent decrease in the intracellular GSH content, with the loss beginning within only a few minutes after infection. The initial loss appears to be a direct effect of the viral interaction with the cell membrane with leakage of GSH from the interior of the cell, through the membrane and into the medium [153] (Fig. 9). The initial loss of GSH is associated with a decreased function of enzymes associated with the maintenance of Na^+/H^+ exchange in the cell membrane. The loss of this exchange pump results in an increase in the concentration of protons (H^+) in the cell and a decrease in the pH of the cell (acidification). It has been demonstrated that acidic conditions favor many viral infections by accelerating the viral fusion process and enhancing viral replication [162–164].

The second step of loss of GSH after viral infection occurs during viral replication. The infected cells show a decrease in GSH and an increase in mixed disulfide formation [153]. It appears that the loss of GSH during viral replication is due to the rapid incorporation of cysteine into the viral gene RNA proteins, which are rich in this amino acid [153,165,166]. Studies showing that an increased oxidative state is critical for viral replication have demonstrated that the administration of the antioxidant dithiothreitol (DTT) to maintain high GSH levels in the cells resulted in a decrease in virus production. It was theorized that the higher GSH levels resulted from inhibiting the formation of mixed disulfides and results in the production of inactive virus by inhibiting the folding process of viral proteins [131]. Decreasing

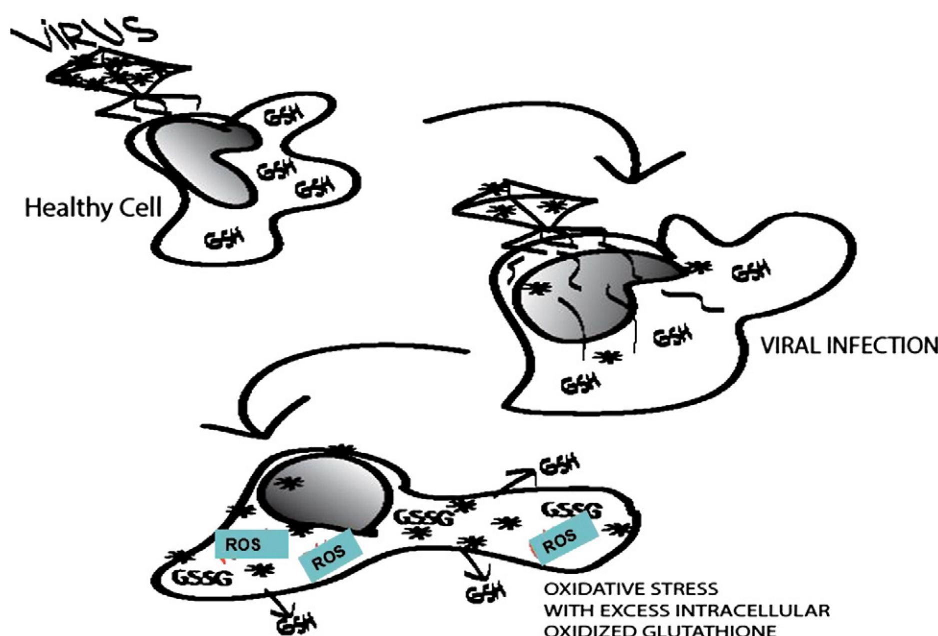

Fig. 9. Depicts that reduction and loss of GSH appears to be a direct effect of the viral interaction with the cell membrane with leakage of GSH from the interior of the cell, through the membrane and into the medium.

GSH with a mixed disulfide-forming agent resulted in an increase in viral replication [153].

Adding the unformulated antioxidant GSH, in the reduced state to cell culture has been shown to decrease viral reproduction in several viral models including both RNA virus (Sendai virus) [167] and DNA virus, Herpes simplex virus-1 (HSV-1) [152] . GSH sensitivity has been also demonstrated infection with Sendai (parainfluenza) virus in African green monkey kidney (AGMK) cells [167], HSV-1 replication in VERO cells, AGMK [152], HIV in monocytic U1 cell line [168], cytomegalovirus (CMV) on rat heart endothelial cells [169], influenza virus on both Madin–Darby canine kidney cells and human small airway epithelial cells [155], and rhinovirus in respiratory epithelial cells [170].

Studies *in vivo* have also documented the ability of GSH to inhibit viral replication and reduced tissue damage. GSH was shown to inhibit CMV replication and vascular tissue damage *in vivo*, after CMV infection in rats [169]. This effect was most noted in the rat heart endothelial cells, which have higher GSH levels than the other tissues examined [169]. The ability of GSH to inhibit influenza virus has also been demonstrated *in vivo* using a mouse animal model. The study shows that the inclusion of unformulated GSH in the drinking water of the animals resulted in a decreased viral titer in the trachea and lung [155].

Studies of the mechanism of GSH's inhibition of viral replication appears to confirm the earlier theory that maintaining GSH levels inside the cells is able to directly inhibit viral proteins crucial for virus replication [167]. The mechanism of GSH inhibition of viral replication in Sendai and influenza virus appears to be different from the mechanism seen with GSH inhibition of HIV virus. In HIV virus, the mechanism is thought to be related to the ability of GSH to prevent oxidative stress and the upregulation of the cellular transcription factor NFκB, which enhances HIV-transcription and replication [171].

In RNA viruses such as Sendai virus, GSH inhibits viral replication by inhibiting the late stages of viral replication [167]. In the HSV-1 model substantial inhibition of viral production was observed even when treatment with GSH was started at 3, 6 and 24 h post infection [152]. The inhibitory activity of GSH is found at intracellular levels similar to those found physiologically. In the HSV-1 virus and in HIV virus, GSH was found to inhibit a glycoprotein, which is essential for the formation of the viral envelope, while the expression of other virus proteins was less affected [130]. GSH also shows substantial protection against the formation of viral matrix protein in MDCK cells [155]. The influenza spike glycoprotein, hemagglutinin (HAO) is critical for enveloped viruses to invade host cells [163]. It has been shown that the correct folding for formation of the HAO protein is dependent on the presence of an oxidized environment, which allows the formation of disulfide bonds in the HAO monomer [172]. The addition of a strong reducing agent, *dithiothreitol* DTT, to the developing virus prevents production of HAO. Viral replication is blocked, because the glycoprotein monomer was not completely trimerized for its final form and it was not exported out of the endoplasmic reticulum (ER) of the host cell [172]. Maintenance of GSH leading to the blockade of glycoprotein folding and interruption of viral production has been shown in vesicular stomatitis virus G protein [173,174] and Semliki forest virus [172]. The common feature in these glycoproteins is an abundance of cysteine, which can create disulfide bonds in an oxidized state. While the maintenance of the oxidation state in the ER appears to be energy dependent [175], the oxidation state of the ER can be rapidly modified by the addition of external antioxidants [172]. There is some suggestion that the effect of GSH added to cell cultures could occur extracellularly and at the same time, the incidence of apoptosis after addition of GSH to cell culture is decreased and an increase in cell viability is noted [155]. The addition of liposomal reduced glutathione (LRG) to a cell culture of T-lymphoblastoid cells (HSB-2) infected with the Human Herpes Virus-6 (HHV-6) has shown similar positive effects in maintaining cell viability (unpublished data).

Oxidation stress and decreased intracellular GSH appears to play a role in the replication of many viruses. The activity of GSH in limiting viral replication such as influenza has been established in both *in vitro* and *in vivo* models. It appears that further research in regard to the use of GSH in the management of viral infection appears warranted.

8. Protozoan parasites

8.1. GSH in kinetoplastids: trypanosoma and leishmania

Protozoan, Kinetoplastid parasites utilize a compound called trypanothione [$T(SH)_2$] as their main thiol antioxidant [for reviews see 176–177, 179–180]. $T(SH)_2$ is composed of 2 molecules of GSH and one molecule of spermidine, and is synthesized in a two-step process unique to kinetoplastids (Fig. 10A–B) [176–180]. Both reactants,

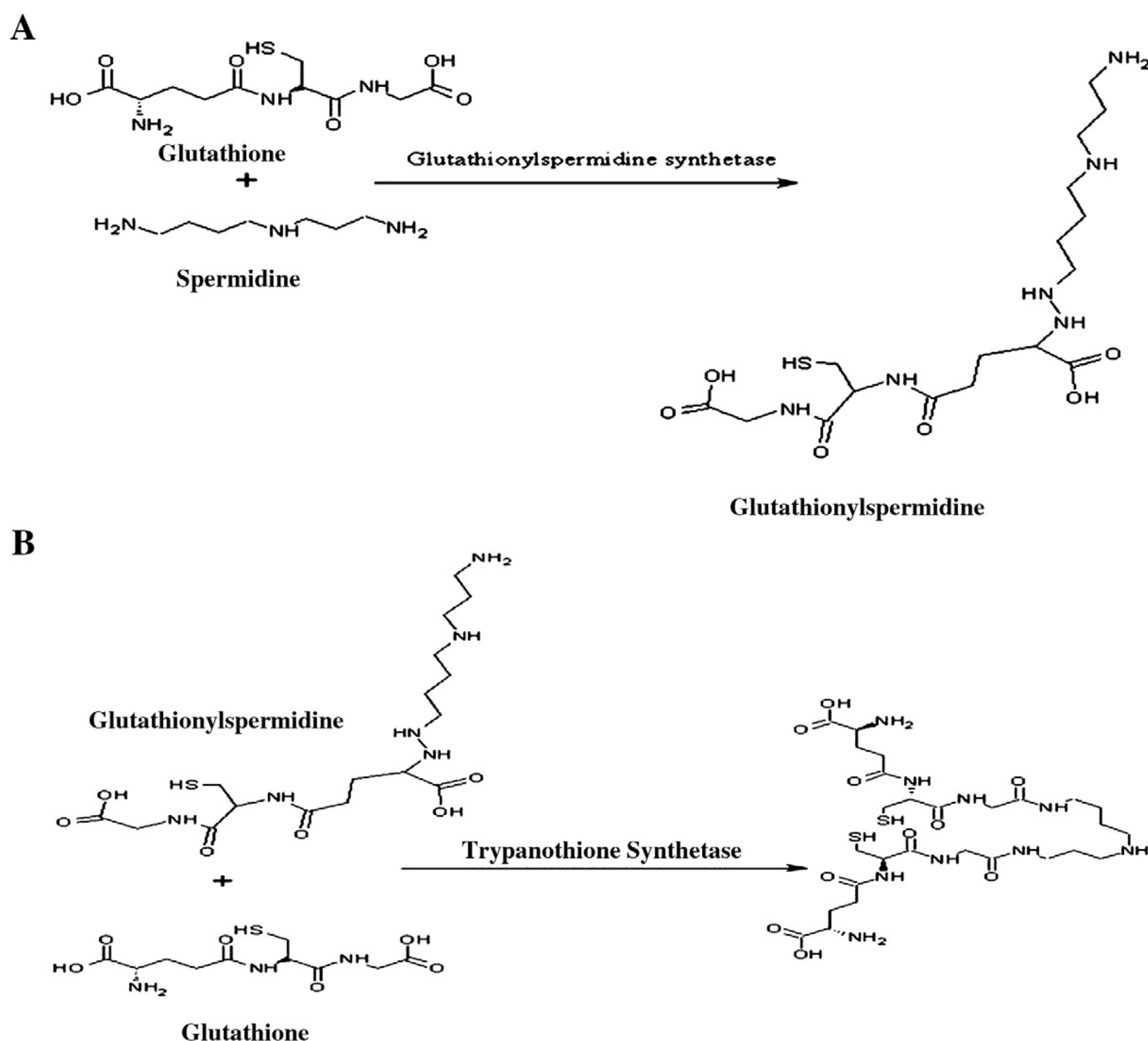

Fig. 10. A: Depicts the first step in the reaction, when the enzyme Glutathionylspermidine Synthetase (GspS) covalently links spermidine with one molecule of GSH to produce glutathionylspermidine. B: Depicts the subsequent step, where the enzyme Trypanothione Synthetase (TryS) attaches a second molecule of GSH to the free end of the spermidine molecule, producing trypanothione.

spermidine and GSH are produced in the parasite via mechanisms common to both parasite and host cells [176–181]. In fact, BSO, a compound shown to inhibit the synthesis of GSH in mammals has been shown to inhibit the synthesis of GSH in trypanosomes [181–183]. In the first step reaction, the enzyme Glutathionylspermidine Synthetase (GspS) covalently links spermidine with one molecule of GSH to produce glutathionylspermidine (Fig. 10A) [176,177,179]. In the subsequent step, the enzyme trypanothione synthetase (TryS) attaches a second molecule of GSH to the free end of the spermidine molecule, producing trypanothione (Fig. 10B) [154,155,157]. $T(SH)_2$ maintains cellular redox balance by undergoing oxidation and reduction in a manner similar to GSH. $T(SH)_2$ detoxifies ROS by donating hydrogen ions from its sulfur-containing cysteine residues, and in the process forms a disulfide bond. Reduction of $T(SH)_2$is carried out by the enzyme trypanothione reductase (TR), which utilizes NADPH as a co-factor, similar to GSR (Fig. 11) [176,177,179]. $T(SH)_2$ also has the ability to directly reduce GSSG, providing new GSH molecules for $T(SH)_2$ production [184].

8.2. GSH as a potential drug target in kinetoplastids

Although GSH is not used directly by kinetoplastid parasites to maintain redox balance, it is a key component of trypanothione, and is therefore integral to their survival. A study dating back to the 1980s demonstrated that inhibiting the production of GSH in *trypanosoma* via administration of BSO can improve the survival time of mice infected with *T. brucei* and with longer duration treatments bring *T. brucei* to levels undetectable in the blood [181]. BSO has also been demonstrated to increase the efficacy of Nifurtimox and Benznidazole in mice, two drugs used in the treatment of acute trypanosomiasis [182,183]. As these drugs are thought to increase oxidative stress in *trypanosoma* [186,187], the addition of BSO will limit the production of GSH and by extension, $T(SH)_2$, exacerbating the oxidative stress brought on by Nifurtimox and Benznidazol [182,183]. Treatment with BSO allows for the administration of lower doses of anti-trypanosomal drugs with the same effect as higher doses. As anti-trypanosomal are associated with severe side effects, the ability to lower dosages may improve patient outcomes [182,183,185].

8.3. GSH in Plasmodium

Many organisms which are parasites of humans utilize GSH as a thiol-containing antioxidant. In particular, *Plasmodium falciparum*, as well as flat and round worm parasites utilize GSH. *Plasmodium* as well as its host RBCs, are exposed to enhanced oxidative stress during RBC infection of *Plasmodium* due to the parasite's digestion of hemoglobin, as well as attack by the host immune system [188–191]. This unavoidable byproduct of the parasite's life-cycle makes the scavenging of ROS by GSH an absolute necessity for its survival. *P. falciparum* is

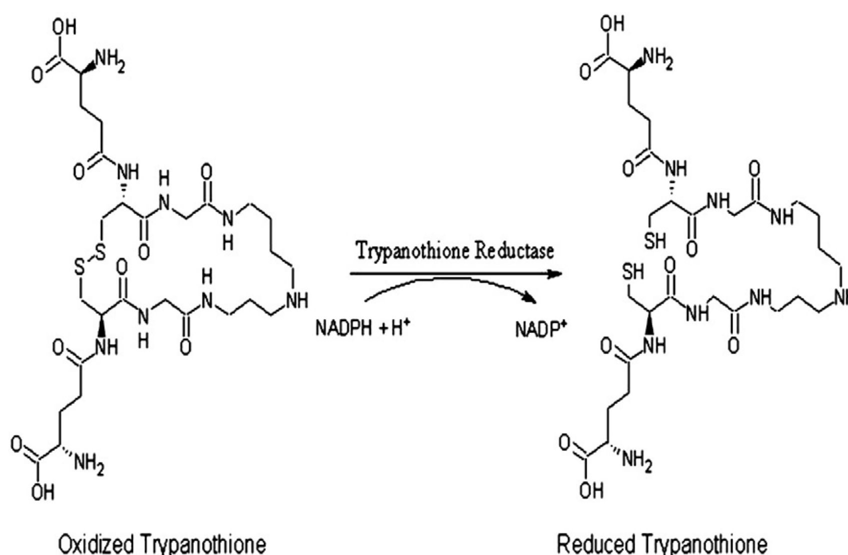

Fig. 11. Depicts reduction of $T(SH)_2$is carried out by the enzyme Trypanothione Reductase (TR), which utilizes NADPH as a co-factor, similar to GSR.

capable of *de novo* synthesis of GSH as well as reduction of GSSG to GSH in the manner carried out in its human host cells [191–195]. Infection by *Plasmodium* is known to exert high oxidative stress on the host erythrocyte [191,196], as infection with *Plasmodium* causes loss of GSH through efflux of GSSG [176,188,191,196,197]. The parasite is able to supply the host cell with GSSG which can be reduced by the host to GSH [196]. In a recent study *P. berghei*, was demonstrated to deplete GSH in the brain, a highly a metabolically active organ with large GSH requirements [198]. The study also demonstrated that treatment with high doses of GSNO afforded protection from cerebral malarial infection, probably due to reduction of inflammation in the brain [198].

8.4. GSH as a potential drug target in Plasmodium

Plasmodium digests hemoglobin for nutrients, and in the process produces a toxic byproduct known as ferriprotoporphyrin IX or hemin (FPIX), which is detoxified to an insoluble polymer (Fig. 12) [199,200]. Quinolines are widely used antimalarial agents which interfere with the detoxification of hemin, a toxic byproduct of hemoglobin digestion conducted by *Plasmodium*. Quinolines act by forming a complex with FPIX which blocks polymerization [201,202]. These Quinoline–FPIX conjugates have toxicity to *Plasmodium* that is similar to free FPIX [203,204]. GSH can directly detoxify FPIX through the formation of GSH–FPIX conjugates via glutathione s-transferase activity (GST) [205]. This mechanism bypasses the activity of quinoline drugs. In addition, resistance of *Plasmodium* to chloroquine has been associated with increased GSH production [206]. Recently, several plant extracts have been identified which inhibit the enzymatic activity of GST in *Plasmodium* which may increase the efficacy of quinoline drugs in quinoline-resistant strains of malaria [207]. Further, it has been shown that administration of BSO to chloroquine resistant strains along with concurrent treatment with chloroquine has shown abrogation of resistance [206]. BSO has also been demonstrated to have a Plasmodicidal effect by increasing the need for GSH in infected RBCs [191]. This indicates that inhibition of GSH production by *Plasmodium* may be a potential drug target (Table 1).

The structure of plasmodial GSR has been analyzed and found to have a key difference from mammalian host GSR. The shape and internal chemistry of a cavity present at the homodimer interface of GSR from *P. falciparum* is much different from its human counterpart. Specifically, the interior of the cavity in human GSR is negatively charged, while the interior of the cavity in Plasmodial GSR is neutral [208]. This cavity is thought to be the site of interaction between Plasmodial GSR and the anti-malarial compound methylene blue (MB) [209]. It is believed that the differential geometry and internal chemistry of the cavity is the reason why methylene blue has a greater affinity for plasmodial GSR than human GSR [209]. MB is also believed to interfere with the activity of GSR by indirectly removing available NADPH which is required for GSR function. MB is reduced by $FADH_2$ to generate leucomethylene blue (LMB) and FAD. LMB can then be oxidized by oxygen, regenerating LMB which is again reduced by $FADH_2$. This cycle of oxidation and reduction uses up available $FADH_2$, while MB is constantly regenerated. NADPH which is required for the normal function of GSR is also required for the reduction of FAD to $FADH_2$ and is used up by the constant reduction of FAD induced by MB. Due to the unavailability of NADPH for GSR function, oxidative stress is increased in *Plasmodium* causing a plasmodiocidal effect [210]. A recent study has also shown that MB can bind to hematin in a manner similar to quinolines, inhibiting its polymerization and leading to toxicity [211].

9. Metazoan parasites

9.1. GSH in nematodes (round worms)

The primary antioxidant in nematode parasites is GSH, and its production and use is similar to that found in other organisms [176,212–214]. Nematodes possess a full suite of GSH synthesis and metabolism synthesis enzymes [212–222]. Nematode parasites produce a secreted from of GST which aids the organism in the evasion of the host immune system [217–223]. GSTs are thought to detoxify antihelminthic drugs by conjugating them to GSH, as well as neutralize the byproducts of lipid peroxidation formed from free radicals produced by the host immune system in response to infection [220–224]. In hook worms GST is believed to detoxify heme during the digestion of hemoglobin in a fashion similar to *Plasmodium* (described earlier) according to a study conducted in *Necator americanus* [225]. Recently, the GSTs of nematodes have been investigated as a potential drug target with promising results [226–230].

9.2. GSH as a potential drug target in nematodes

Due to the use of GSH as a reducing agent for antihelminthics, ROS, and byproducts of lipid peroxidation in nematodes, GSH synthesis would seem to be an attractive drug target. Several studies have investigated the efficacy of inhibiting GSH production to kill nematode parasites with mixed results [231–233]. BSO has been demonstrated to inhibit the production of GSH in *Ascaris* [231]. Curcumin has been

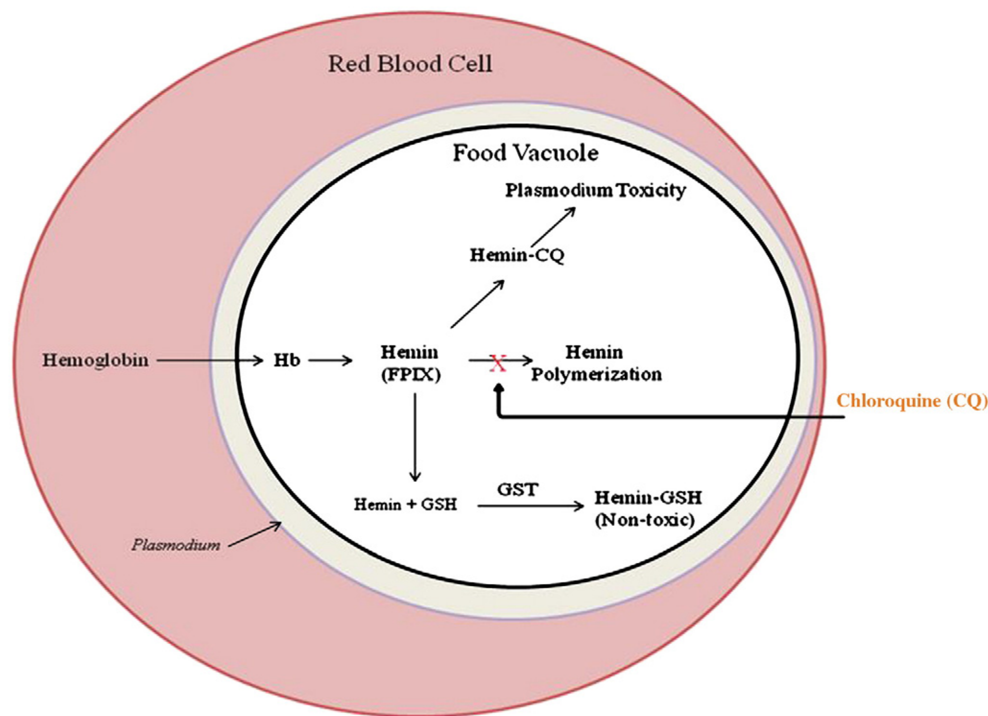

Fig. 12. *Plasmodium* digests hemoglobin for nutrients, and in the process produces a toxic byproduct known as ferriprotoporphyrin IX or hemin (FPIX), which is detoxified to an insoluble polymer.

shown to induce GSH depletion and apoptosis in *Setaria cervi* [232]. Another study conducted in several different nematode parasites demonstrated depletion of GSH with administration of xylofuranosylated diaminoalkanes, accompanied by a complete loss of motility and varying degrees of killing on adult and egg forms. A sterilization effect was also observed on adult female forms of the parasites [233]. A third study however, demonstrated increased resistance to antihelmintic drugs with the depletion of GSH [234]. These conflicting studies indicate that the role of GSH in nematode parasites is not yet fully understood and should be an area of focus for further study (Table 1).

9.3. GSH in platyhelminths (flat worms)

Platyhelminth parasites rely heavily on GSH in the maintenance of their cellular redox state. Flat worm parasites possess a linked thioredoxin (Trx) and GSH system [235,236]. Trx is a protein, which contains a disulfide bond, and it is used in the maintenance of the cellular redox state of many organisms, including humans. Trx is maintained in a reduced state in a manner similar to GSH. Parasitic flat worms lack traditional GSH and thioredoxin reductase enzymes, and instead produce a hybrid thioredoxin glutathione reductase (TGR) [235,236]. *Shistosoma mansoni* as recently been revealed to produce a phytochelatin synthase enzyme (PCS) [237]. This enzyme catalyzes the production of phytochelatin which utilizes GSH to sequester and detoxify heavy metals in the parasite [237].

9.4. GSH as a potential drug target in platyhelminths

As TGR and PCS are not present in the hosts of parasitic platyhelminths, they are enticing drug targets for chemotherapy. Several inhibitors of shistosomal TGR have recently been identified, and are currently being studied as antihelminthic therapies [238–243]. Recently conducted studies indicate that GST may play a role in the detoxification of antihelmintics and lipid peroxidation products in platyhelminths in a manner similar to that observed in nematodes [244–246]. Inhibitors of GST in parasites are also being studied as potential drug targets [247].

9.5. GSH in fungal infections

Fungi possess a complete suite of GSH synthesis enzymes, similar to those found in humans, including GCL, GSH synthase (GSS), and GSR [248]. Recently, benzaldehydes have been identified as agents that inhibit the activity of fungal GSR in Aspergillus [249,250]. In addition, GSH has been shown to decrease the toxicity of gliotoxin from *Aspergillus fumigatus*, increasing neuronal and astrocyte viability after gliotoxin exposure [251]. The inhibition of fungal GSR by benzaldehydes enhances the potency of antifungal drugs, lowering their minimum inhibitory (MIC) and minimum fungicidal (MFC) concentrations [249,250]. This synergistic effect has great potential for chemotherapeutic treatment in fungal infections as many antifungal drugs are not well tolerated. The ability to administer lower doses of antifungal medications while maintaining fungicidal activity would greatly enhance the efficacy of antifungal therapy (Table 1).

Several pathogenic fungi, including *Histoplasma capsulatum* the causative agent of histoplasmosis employ GSH and GGT as a means of acquiring iron which is required for survival of the fungus within the host. These fungi secrete a form of GGT which breaks the γ-glutamyl bond of GSH to produce cysteinylglycine, which then reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) which can then be utilized [250,252–254]. As the acquisition of iron is essential for survival of these pathogenic fungi, fungal GGT presents a potential target for chemotheraputic agents (Table 1).

10. GSH- an immuno-modulatory agent

GSH's effect is both direct (antimycobacterial activity) and indirect (immune enhancing activity). GSH's structure resembles that of penicillin, and has been termed “glutacillin.” GSH also serves as a carrier for NO within the macrophage thereby serving directly as an antimycobacterial peptide.

The indirect antibacterial effect appears to be broad in scope and is concentration dependent. GSH-enhancing agents appear to enhance the cellular immune response while antagonizing the humoral

Table 1
Significance of GSH in humans and pathogenic microbes.

Organism	Principal thiol antioxidant	Host/Pathogen significance	Significance of GSH in the human host or pathogenic organism
Apicomplexa	GSH	Pathogen	GSH protects plasmodium from the enchanced oxidative stress inherent to the parasite's life-cycle. *Plasmodium* utilizes GSH to detoxify FPIX, by passing quinoline activity
Kinetoplastida	Tripanothione	Pathogen	Kinetoplastids synthesize trypanothione from GSH. Inhibing the synthesis of GSH in the pathogen enhances the effects of anti-trypanosomal drugs.
Filaria	GSH	Pathogen	Nematodes utilize GSH to detoxify antiheminthics. Hook worms utilize GSH to detoxify heme similar to *plasmodium*.
Platyhelminths	GSH	Pathogen	Platyhelminths utilize GSH to detoxify antihelminthics.
Mycobacteria	Mycothiol	Host	GSH has direct anti-mycobacterial activity. GSH enhances the intracellular killing of M. tb in macrophages. GSH enhances the ability of NK cells and T cells to control intracellular *M. tb* infection. GSH enhances the production of TH_1 cytokines.
HIV	N/A	Host	GSH is depleted in HIV infected individuals. GSH enhances activity of the immune system and favors the production of TH_1 cytokines.

response. This is accomplished mainly by favoring cytokine expression profiles of TH1 CD4+ cells and enhancing the functions of NK cells.

Decreased levels of GSH impairs the functions of immune cells and this effect appears to be central to the pathogenesis of HIV offering another explanation for rising incidence of *M. tb* among individuals with HIV infection. It is therefore not surprising that the reemergence of TB has occurred in developing countries that have the highest global prevalence of HIV+ cases.

Recent developments have shown that this is not much of a coincidence, suggesting even that HIV and TB form a partnership during times of co-infection. The partnership formed results in enhanced morbidity and mortality. The understanding that oxidative stress is integral to this mechanism has positioned the GSH system as a potential therapeutic and an immunomodulatory agent (Table 1).

Treatment of HIV-TB co-infected individuals is challenging because highly active antiretroviral therapy (HAART) and antituberculosis drugs commonly interact; hence, alternative regimens have gained attractiveness. Ultimately suggesting a new indication for GSH-enhancing agents, which till previously had three uses: to break up mucous plugs in cystic fibrosis, as an antidote for acetaminophen overdose, and as an adjunct treatment for contrast-induced nephropathy. Therefore, increasing the levels of GSH in lungs by administering GSH-enhancing agents should help reestablish the immune functions in individuals with HIV and or *M. tb* infection. Knowledge of the mechanisms of GSH regulation and balance between the release and expression of pro- and anti-inflammatory mediators could lead to the development of novel therapies based on the pharmacological manipulation of the production as well as gene transfer of this important antioxidant in lung infection and inflammation (Table 1).

10.1. Current and future medical use of NAC

NAC, a GSH enhancing agent has been used for over 30 years in the United States, initially limited to the setting of acute acetaminophen overdose. Within the last 15 years, NAC has been used in the prevention of contrast-induced nephropathy in patients with renal insufficiency (GFR<60); and as a mucolytic in CF and chronic obstructive pulmonary disease (COPD) patients. Currently, NAC is being used as a vasodilator to prevent perioperative atrial-fibrillation (AF). The future of NAC however is moving in a new direction since the advent of recent discoveries elucidating a previously unknown immune modulatory effect of the drug, especially in patients infected with HIV. This discovery offers a potential adjunct treatment option for patients on HAART; improved immune response in patients vaccinated with novel HIV vaccines, and prophylaxis to mycobacterial infection.

Traditionally, NAC can be administered via two routes, either oral or IV. The IV route is indicated during times of hepatic failure in the setting of acetaminophen overdose and has been championed by the United Kingdom since the early 1970s. The typical 20-hour IV protocol involves administration of a loading dose of 150 mg/kg over 60 min, followed by 12.5 mg/kg/h for the next 8 h, and 6.5 mg/kg/h for the remaining 16 h. In the United States, the 72-hour oral protocol is favored. This involves a loading dose of 140 mg/kg followed by 17 doses of 70 mg/kg every 4 h. The dosage is not changed in the setting of activated charcoal use. The effectiveness of either protocol has not been evaluated in a head-to-head clinical trial, furthermore, the effect of NAC in general has not been clinically evaluated vs. no treatment because this would be unethical. Best results are seen when NAC is administered before any elevation of alanine aminotransferase (ALT), studies have demonstrated a 0% mortality rate in this situation; when administered within 8 and 16 h of overdose, mortality rates are 10% and 40% respectively [255].

The prevention of contrast-induced nephropathy in high-risk renal patients have shown mixed results. While 600 mg every 12 h for 2 days duration does transiently decrease serum creatinine (Cr), the effect is only marginal at best (the mean reduction in one study was only 0.85 mg/dL) [256]. Other studies have shown that using a higher dose of NAC, 1200 mg every 12 h for 2 days, had no effect on lowering serum Cr but did decrease adverse events in patients with renal insufficiency treated with contrast compared to patients treated with the lower dose (odds ratio 0.46, confidence interval 0.33–0.64) [257].

Usage in the setting of respiratory disease is beginning to fall out of favor. There have been no studies evaluating the effectiveness in the setting of cystic fibrosis [258]. The mechanism of action in this setting involves liquefaction of mucus and DNA within 1 min, peaking in utility by 10 min [259]. Use of NAC in patients with COPD was evaluate in a large prospective European trial, Bronchitis Randomized on NAC Cost–Utility Study (BRONCUS), there was no difference in number of exacerbations or change in lung function vs placebo over a three year period [260]. It was proposed that NAC may have a role in the treatment of patients with idiopathic pulmonary fibrosis (IPF) based on observations of low GSH in bronchoalveolar lavage (BAL) specimens; however, the most recent study demonstrated a paradoxically increased mortality in patients treated with NAC+ standard of care (azathioprine/prednisone) compared to standard of care alone [261]. Additionally, nebulized NAC induces bronchospasm in a subset of patients, this can be reduced with concomitant beta-2 agonist dosing. For these reasons, most clinicians have greatly reduced their use of nebulized NAC; additionally, nebulized NAC has a rather pungent odor, is relatively expensive, and time consuming to administer.

NAC's effect on endothelial vasodilation has recently been used in the perioperative setting and has been shown to drastically reduce atrial fibrillation in post-operative patients when administered 1 h before surgery and continued for 48 h in patients undergoing coronary artery bypass graphing (CABG) or other valvular surgeries compared to standard of care (5.2% vs. 21.2%) [262]. It appears the mechanism involves increased coronary perfusion when NAC is combined with

Table 2
Consequences of decreased and increased levels of GSH in disease conditions.

Disease	Amount of antioxidant	Consequences and effects
TB	↓ GSH	Increased production of inflammatory cytokines and enhanced growth of *M. tb*
	↑ GSH	Activation of immune cells to produce antimicrobial molecules and enhanced cytolytic activity
Cystic Fibrosis	↓ GSH	Increased levels of activated inflmmatory cells and oxidant molecule
	↑ GSH	Inhibition of oxidant molecule α_1-AT and reduction of inflammatory cells to reduce the damage to the epithelium
Diabetes	↓ GSH	FA oxidation and generation of ROS which damage tissue, increased vascular complications and susceptibility to *M. tb* infection
	↑ GSH	Reduction of ROS and oxidant molecules to reduce damage and control disease
AIDS	↓ GSH	Oxidant induced damage of immune cells and increased susceptibility to *M. tb* infection
	↑ GSH	Decreased levels of ROS and TNF-α, decreased viral load, maintenance and restoration of immune cell functions

nitroprusside; interestingly, the benefit was not observed with nitroglycerin [262].

New efforts are pushing for NAC to be used in patients with HIV, specifically those infected with *M. tb*. NAC is a promising therapeutic secondary to its relatively low cost in oral form, and generally mild side-effects. The most common side-effect of NAC is nausea and vomiting, observed in 33% of patients treated for acetaminophen toxicity (140 mg/kg PO) [263]; while anaphylaxis was observed in approximately 15% of patients given IV NAC (more commonly if given over 15 min rather than 60 min) [264].

HIV is an infection characterized by low levels of total and reduced GSH, oral administration of NAC (1 g/d for 7 days) has been demonstrated to increase intracellular GSH [265]. *In vitro* studies have demonstrated that increased intracellular GSH decreases pro-inflammatory cytokine (IL-1, IL-17, TGF-β) production and up-regulates expression of GSH synthetic enzymes; the effect of which reduces intracellular growth of *M. tb* within cultured monocytes [57]. Additionally, increased GSH levels correlate with increased expression of FASL and CD40L on NK cells from HIV+ patients, the effect of which was shown to independently limit growth of *M. tb* within co-cultured monocytes [62]. These observations suggest the existence of a novel innate immune function centered upon oxidative stress, making NAC an attractive immune modulator favoring cell-mediated (T_H1) immunity. This effect is being studied in mice as a potential way to boost immunogenicity to HIV1-tat vaccine products [92].

10.2. NAC is poised to take center-stage in HIV+ patients co-infected with M. tb

As Venketaraman et al. have demonstrated a clear mechanism and benefit of increased GSH levels that are inversely correlated with intracellular mycobacterial growth. Treatment of TB is difficult in HIV+ patients because many anti-TB drugs commonly interact with retroviral therapy, NAC offers a potential attractive adjunct because evidence suggests that it also reduces mortality in INH and Rifampin induced hepatitis when given at least 100 mg/kg/d [266]. More clinical studies are needed to access the relative utility of NAC use in HIV+ populations and if this provides a protective effect against mycobacterial infection. If protective, NAC could potentially be one of the most cost effective preventive measures in medicine, as HIV/TB co-infection is responsible for staggering morbidity and mortality in the developing world (Table 2).

11. Conclusion

Oxidants, highly reactive free radicals, play a major role in the pathogenesis of a variety of infectious diseases. In the healthy individuals, the oxidant burden is balanced by the local antioxidant defenses. However, both an increased oxidant burden and/or decreased antioxidant defenses may reverse the physiological oxidant-antioxidant balance in favor of oxidants, leading to inflammation and damage to the tissue thereby creating favorable conditions for microbial infections. This concept points to an obvious therapeutic strategy: augmentation of GSH levels to prevent oxidant-mediated tissue damage and as adjunct for treatment of *M. tb* and HIV infection. Studies using reduced GSH, the major pulmonary antioxidant, as a model therapeutic agent demonstrate that GSH can be administered directly to the respiratory epithelial surface by aerosol and is fully functional as an antioxidant both *in vitro* and *in vivo*. In pulmonary diseases such as idiopathic pulmonary fibrosis or following HIV-infection GSH aerosol therapy not only normalize deficient pre-therapy GSH-levels in the lung, but is capable of enhancing the functions of both NK and T cells and regulating the production of pro-inflammatory cytokines and free radicals. This review suggest that use of antioxidants will greatly enhance the immune cell functions to control many bacterial and viral infections, reverse the imbalance between oxidants and antioxidants at the site of oxidant injury and prevent the progressive tissue damage (Table 2).

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